SCIENCE CHINA
Life Sciences

SPECIAL TOPIC
• REVIEW •

May 2012 Vol.55 No.5: 377–385
doi: 10.1007/s11427-012-4322-y

Archaeal chromatin proteins

ZHANG ZhenFeng, GUO Li & HUANG Li*

State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China

Received March 15, 2012; accepted April 17, 2012

Archaea, along with Bacteria and Eukarya, are the three domains of life. In all living cells, chromatin proteins serve a crucial role in maintaining the integrity of the structure and function of the genome. An array of small, abundant and basic DNA-binding proteins, considered candidates for chromatin proteins, has been isolated from the Euryarchaeota and the Crenarchaeota, the two major phyla in Archaea. While most euryarchaea encode proteins resembling eukaryotic histones, crenarchaea appear to synthesize a number of unique DNA-binding proteins likely involved in chromosomal organization. Several of these proteins (e.g., archaeal histones, Sac10b homologs, Sul7d, Cren7, CC1, etc.) have been extensively studied. However, whether they are chromatin proteins and how they function *in vivo* remain to be fully understood. Future investigation of archaeal chromatin proteins will lead to a better understanding of chromosomal organization and gene expression in Archaea and provide valuable information on the evolution of DNA packaging in cellular life.

Archaea, chromatin protein, biochemical properties, structure, post-translational modification

Citation: Zhang Z F, Guo L, Huang L. Archaeal chromatin proteins. Sci China Life Sci, 2012, 55: 377−385, doi: 10.1007/s11427-012-4322-y

All living organisms face the challenging task of packaging extraordinarily long chromosomal DNA into the small space within a cell while permitting ready access to the genetic information in the genome by molecular machineries functioning in DNA transactions. Chromatin proteins serve essential roles in chromosomal organization and compaction as well as in highly regulated expression of genes. The three domains of life have evolved a range of small, basic and abundant DNA-binding proteins believed to be involved in chromosomal organization. This review will focus on these proteins (Figure 1).

Archaeal chromosomes are all circular DNA molecules accompanied occasionally by extrachromosomal genetic elements. Known archaeal genomes range in size from 0.49 (*Nanoarchaeum equitans*) [1] to 5.75 Mbp (*Methanosarcina acetivorans*) [2]. Genomic DNA is negatively supercoiled in mesophilic Archaea but is relaxed to slightly positively supercoiled in thermophilic Archaea [3]. The unique geometry of DNA in thermophilic Archaea, which is not found in any other organisms, may represent a strategy of adaptation of these organisms to growth in hot habitats. It is worth noting that positive DNA supercoiling is consistent with the presence of reverse gyrase, a topoisomerase capable of introducing positive supercoils into DNA at the expense of ATP, in thermophiles [4,5].

The domain Archaea consists of five kingdoms, the Crenarchaeota, the Euryarchaeota, the Korarchaeota, the Thaumarchaeota and the Nanoarchaeota [6]. A number of chromatin proteins from crenarchaea and euryarchaea have been extensively studied. While euryarchaea primarily employ homologs of eukaryotic histones in DNA packaging, crenarchaea appear to have evolved distinctly different strategies for chromosomal organization. Analyses of archaeal chromatin proteins have shed considerable light on the diversity and evolution of DNA packaging mechanisms in living organisms.

*Corresponding author (email: huangl@sun.im.ac.cn)

1 Euryarchaeal chromatin proteins

1.1 Archaeal histones

An archaeal histone was first isolated from *Methanothermus fervidus* in 1990 [7,8]. Over 30 archaeal histone-encoding sequences have now been identified [9]. Genome sequencing shows that nearly all euryarchaea encode archaeal histones. The recent discovery of histone genes in some crenarchaeal genomes [10] suggests that histones are more widely distributed phylogenetically and have a more ancient origin than previously believed (Figure 1).

Archaeal histones exist as dimers in solution and resemble eukaryotic H3 and H4 in structure [11]. Each histone monomer possesses a minimal histone fold including three α-helices separated by two short β-strand loops (Figure 2). Upon binding to DNA, archaeal histone dimers form tetramers and, in some cases, hexamers [12]. The histone tetramer binds approximately 90 bp of dsDNA [13], wrapping it around the surface of the tetramer and thereby constraining negative supercoils at physiological temperature and salt concentrations [14]. It has been shown that tetramerization is critical to the function of archaeal histone since mutations at the dimer-dimer interface impair the ability of the histones to bind DNA [12]. In addition, mutation of some key residues at the dimer-dimer interface alters the direction of supercoiling imposed by the histone tetramers [12].

Many Archaea synthesize multiple histone homologs capable of forming both homodimers and heterodimers. Gene expression for different histone homologs in *Thermococcus zilligii*, *Methanococcus voltae* and *M. fervidus* exhibits different growth phase dependencies [15,16]. It is speculated that the relative abundance of the homo- and heterodimeric forms may modulate the nature of chromosomal organization during the different growth phases [17]. Histones from *Methanopyrus kandleri* and *Halobacterium salinarum*, referred to as HMk homologs, are composed of two tandemly fused histone folds separated by a linker region [18,19] (Figure 2A). These proteins are thus obligate pseudo-heterodimers.

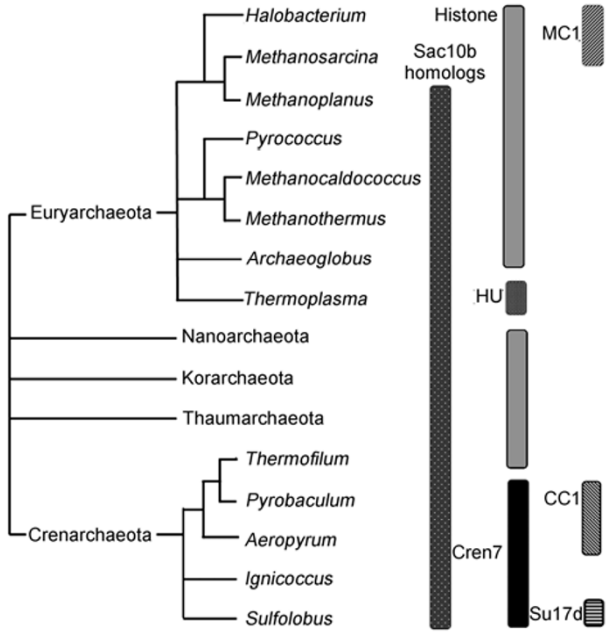

Figure 1 Phylogenetic distribution of proposed archaeal chromatin proteins. Representative archaeal species are indicated.

Genetic analysis of archaeal histones was first applied to *Methanococcus voltae*, the genome of which encodes three histone-folding proteins (i.e., HstA, HstB and HMvA) and a single Sac10b homolog [20]. Deletion of any individual protein did not affect viability, demonstrating that the chromatin proteins probably have overlapping functions. Only one mutant strain, ΔHstA, exhibited a slightly decreased growth rate. However, the abundance of a variety of cellular proteins from the four mutants varied, indicating a pleiotropic effect on gene expression. The requirement for an archaeal histone was then evaluated in *Methanosarcina mazei* Göl by insertional disruption of the only histone, HMm [21]. Loss of HMm was not lethal but resulted in mutants that have impaired ability to grow on methanol and trimethylamine, suggesting that the archaeal histone is dispensible. Transcription levels for approximately 25% of all *M. mazei* genes decreased in the mutant strain, indicating that HMm probably plays important roles in transcriptional activation.

In Eukarya, modulation of chromatin structure through physical and covalent modification of core histones is an integral part of DNA transactions such as transcription, DNA repair and replication [22]. The N-terminal tails of histones, thought to be important for mediating inter-nucleosome interactions, are sites for extensive post-translational covalent modifications, including methylation, acetylation, phosphorylation and ubiquitination [22]. Acetylation and methylation of histone tails are key events in the regulation of eukaryotic genetic processes [22,23]. Unlike their eukaryal counterparts, archaeal histones lack the N- and C-terminal extensions [22] (Figure 2). To date, only one of the components involved in eukaryal histone modifications, i.e., the histone acetyltransferase Elp3, has been shown to have homologs in Archaea by genome sequence analysis [8,24,25]. However, no acetylations or other forms of modification have been demonstrated in archaeal histones.

1.2 Other euryarchaeal chromatin proteins: HTa and MC1

HTa was isolated from *Thermoplasma acidophilum* about 30 years ago, and, based on sequence similarity analysis, was proposed to be a member of the bacterial HU protein family [26]. HU is one of the most abundant bacterial chromatin proteins [27]. Hta is found to be restricted to the genera of *Thermoplasma* and *Ferroplasma*. Intriguingly,

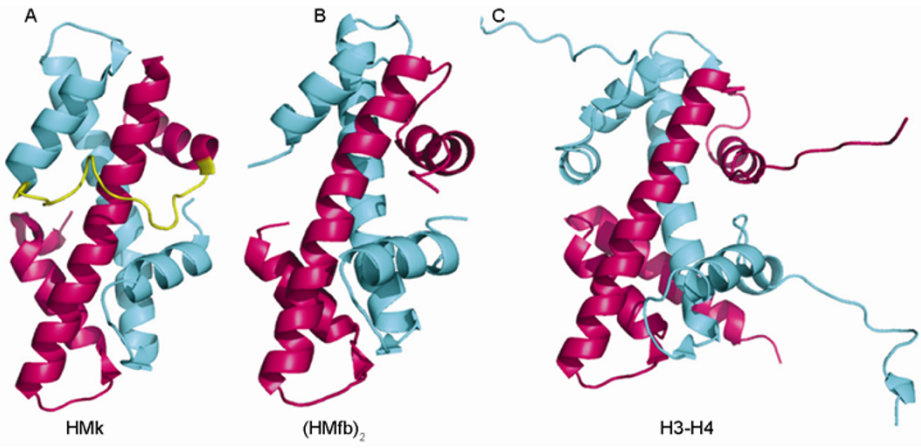

Figure 2 A comparison of crystal structures of archaeal and eukaryotic histones. A, Structure of HMk. The two histone folds are shown in cyan and magenta, respectively, and the linker region between them is highlighted in yellow. B, Structure of $(HMfb)_2$. The two subunits are shown in cyan and magenta, respectively. C, Structure of a H3-H4 dimer. H3 is shown in magenta and H4 in cyan. The figures were generated with PyMol using the PDB coordinates 1F1E, 1BFM and 1AOI, respectively.

these organisms are the only euryarchaea known to lack histones. HTa has been shown to raise the melting temperature of bound DNA and to cause severe DNA bending [26]. The physiological function of the protein is unclear but, based on studies on bacterial HU proteins, HTa may participate in chromosomal compaction and gene regulation.

Methanosarcina and *Methanothrix* encode an abundant DNA-binding protein, termed MC1, in addition to a single archaeal histone [28]. MC1 family members are also predicted to be present in *Halococcus*, *Halobacterium* and *Haloferax* [29]. Sequence comparison reveals no relationship between MC1 and other known chromatin proteins. MC1, estimated to be present at ~1 monomer per ~170 bp in the *Methanosarcina* cell [28], binds preferentially to negatively supercoiled DNA and to four-way Holliday structures with a binding size of ~11 bp per monomer [30]. Binding by MC1 results in DNA bending and negative DNA supercoiling, and protects the DNA against radiolysis and heat denaturation [28]. MC1 was shown to stimulate *E. coli* RNA polymerase activity *in vitro* at low protein to DNA ratios, and inhibit the transcription at higher ratios [31].

2 Crenarchaeal chromatin proteins

2.1 Sul7d

Hyperthermophilic Archaea of the genus *Sulfolobus* synthesize large amounts of small, basic DNA-binding proteins [32,33]. Among these proteins, members of the 7-kD protein family are the most abundant. The 7-kD proteins were first isolated from *S. acidocaldarius*, which produces five species, designated Sac7a-e in order of increasing basicity [32]. Highly similar homologs have been found in all *Sulfolobus* species as well as in the related *Metallosphaera sedula*, but not in other Archaea [34]. These proteins, collectively known as Sul7d proteins, have been extensively studied over the past three decades. The proteins bind double-stranded DNA as monomers and with little sequence specificity [35]. Binding by the Sul7d proteins raises the Tm of DNA by as much as 40°C [35]. Moreover, Sul7d also facilitates the reannealing of complementary single strands of DNA at high temperature [36]. Fluorescence titrations and gel shift assays have shown that the affinity of the Sul7d proteins for double-stranded DNA is in the micromolar range in low salt [35,37,38]. However, different estimates of the binding size (3–6.6 base pairs) were obtained in these studies [35,37,38]. Sul7d is capable of constraining negative DNA supercoils [39,40].

Structures of several Sul7d proteins alone and in complex with DNA have been resolved [41–44]. These proteins show a SH3-like organization with a β-barrel fold. In the protein-DNA complexes, Sul7d binds dsDNA in the minor groove (Figure 3). Two hydrophobic residues Val26 and Met29 are intercalated between base pairs, introducing a sharp single-step kink (~60°) in the double helix [42,43]. Unwinding and bending of the DNA as the result presumably provide the structural basis for the ability of Sul7d to constrain negative DNA supercoils *in vitro* [42,43].

The Sul7d proteins from both *S. acidocaldarius* and *S. solfataricus* are monomethylated at selected lysine residues to different extents in a strain-dependent manner [45–48]. The extent of the lysine methylation increases *in vivo* with increasing growth temperature [45]. These observations point to the functional relevance of Sul7d methylation. However, methylation does not affect the binding affinity of Sul7d for DNA, probably because the lysine residues that undergo methylation are not located at the protein-DNA interface [42].

Sul7d homologs make up nearly 5% of the cellular protein [44]. Their abundance, together with their ability to bind DNA without sequence specificity and to constrain DNA in negative supercoils, suggests that they may play an

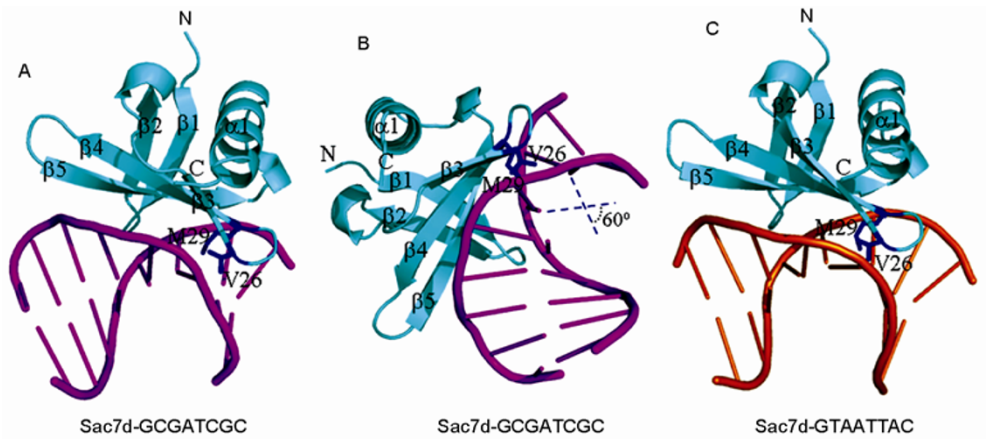

Figure 3 Structure of Sac7d bound to DNA. A, Structure of Sac7d-GCGATCGC (PDB code: 1AZP). The duplex DNA is bent by 70° as the result of its interaction with Sac7d in the minor groove. The intercalated residues are shown as sticks in blue (Val26 and Met29). B, The side view of the structure in panel A. The sharp kink (~60°) between A3 and A4 step is marked. C, Structure of Sac7d-GTAATTAC (PDB code: 1AZQ). The figures were generated using PyMol.

important role in DNA packaging and chromosomal organization *in vivo*. This is consistent with the observation that Sul7d compacts relaxed or positively supercoiled DNA [40,49]. Given their effect on DNA geometry, Sul7d proteins likely serve a role in duplex stabilization. Transient single-stranded DNA bubbles probably form more readily and extensively at temperatures suitable for the growth of hyperthermophiles [50]. These single-stranded regions in DNA are potential sites for the initiation of abnormal or detrimental cellular processes. Sul7d may protect the chromosomal DNA of *Sulfolobus* from undergoing local denaturation at high growth temperatures through direct protein-DNA interactions as well as by its effect on the superhelicity of protein-free DNA regions.

Interestingly, Sul7d is capable of reversing thymidine dimers through a photoreactivation-type reaction, protecting DNA from UV-induced damage. Irradiation of Sul7d leads to oxidation of Trp24, a conserved tryptophan residue located at the DNA binding interface. If the tryptophan residue is close to a thymidine dimer, electron transfer occurs, leading to oxidation and thus repair of the thymidine dimer [51]. Although this process is much less efficient than photoreactivation mediated by photolyases, the very high *in vivo* level of Sul7d may ensure that Sul7d-mediated repair is physiologically relevant.

2.2 Cren7

Cren7 is the only chromatin protein known to be conserved at the kingdom level in the Crenarchaeota. The protein was recently isolated from *S. shibatae* [33]. pBLAST results show that all genome-sequenced crenarchaea encode Cren7 homologs except for *Thermophilum pendens* Hrk5 (Figure 1 and 4A). Intriguingly, *T. pendens* Hrk5 encodes an archaeal histone, whereas all Cren7-encoding crenarchaea do not [27,33,52]. Therefore, it is tempting to speculate that Cren7 serves a function in crenarchaea similar to that of archaeal histones in euryarchaea, and that the crenarchaeal branch in Archaea shares a common strategy in chromosomal organization.

Cren7 from *S. shibatae* is a polypeptide of 60 amino acid residues and has an isoelectric point of 9.87 [33]. The protein accounts for ~1% of the total cellular protein. Native Cren7 from *S. shibatae* is multiply methylated at some of its 12 lysine residues [33]. Unlike what has been shown for Sul7d, methylation occurs at several lysine residues located at the DNA binding surface (e.g., K16 and K31) [33]. Modification at these sites may affect the interaction of the protein with DNA. The protein binds preferentially to double-stranded DNA *in vitro* and is associated with genomic DNA *in vivo*. Cren7 protects dsDNA from thermal denaturation by increasing its melting temperature by up to ~29°C. Cren7 constrains negative DNA supercoils about twice as efficiently as Sul7d. As revealed by NMR, Cren7 adopts an SH3-like fold [33]. It interacts with duplex DNA through a β-sheet and a long flexible loop between the β3- and β4-strands. In the crystal structures of Cren7 from *S. solfataricus* in complex with two short double-stranded DNA fragments, the protein binds in the minor groove of DNA and causes a single-step sharp kink in DNA (~53°) through the intercalation of the hydrophobic side chain of Leu28 [53] (Figure 4B). Loop β3-β4 of Cren7 undergoes a significant conformational change upon binding of the protein to DNA, suggesting its critical role in stabilizing the protein-DNA complex.

Cren7 resembles Sul7d in structure [33,53]. However, the two proteins share no significant similarity at the amino acid sequence level. Both Cren7 and Sul7d appear to be functional since they are both synthesized in large quantities in the cell. Thus, the question arises as to why they are both needed. A structural comparison between the DNA-binding surfaces of the two proteins may offer some clues (Figure

4C and D). First, Cren7 possesses a long loop between β3 and β4 in the DNA-binding surface, which contains four DNA-interacting residues and covers about two base pairs, while Sac7d, a Sul7d protein from *S. acidocaldarius*, has a small hinge in the corresponding region (Figure 4C). Second, the sheet formed by the β1- and β2-strands in Cren7 does not interact with DNA (Figure 4D). By contrast, Lys7, Tyr8 and Lys9, which are located in the corresponding region in Sac7d, interact with the deoxyribose of the DNA backbone. Third, a single residue (Leu28) in Cren7 is intercalated between the base pairs of the bound DNA, generating a sharp DNA kink. In comparison, a similar DNA deformation is caused by intercalation of two residues (Val26 and Met29) in Sac7d [42,43]. Val36, which corresponds to Met29 in Sac7d and is highly conserved in Cren7, contacts the deoxyribose of the DNA (Figure 4C). These observations reinforce the notion that Cren7 and Sul7d serve distinct functions in chromosomal organization and regulation of gene expression.

2.3 CC1

Analysis of genome sequences has revealed no homologs of genes encoding single-stranded DNA binding protein (SSB) in organisms of the order Thermoproteales. A search for proteins capable of ssDNA binding led to the identification of CC1, a small basic protein of primarily beta-sheet organization [54]. CC1 shows nearly identical affinities for ssDNA and dsDNA, but binds to the former in a highly cooperative manner. A CC1 monomer binds approximately six base pairs [55]. The ability to bind strongly to ssDNA separates CC1 from Sul7d or Cren7, which binds ssDNA poorly [55]. Although CC1 is restricted to the order Thermoproteales and *Aeropyrum pernix*, its beta sheet-rich structural feature is shared by both Sul7 and Cren7, an observation suggestive of the evolutionary relationship among various crenarchaeal chromatin proteins despite their low amino acid sequence similarity.

3 The Sac10b protein family (Alba)

A small, abundant DNA-binding protein, denoted Sac10b, was first isolated from *S. acidocaldarius* in the mid-80s [56]. Later, homologs of Sac10b were found to be highly conserved among Archaea and make up the Sac10b protein

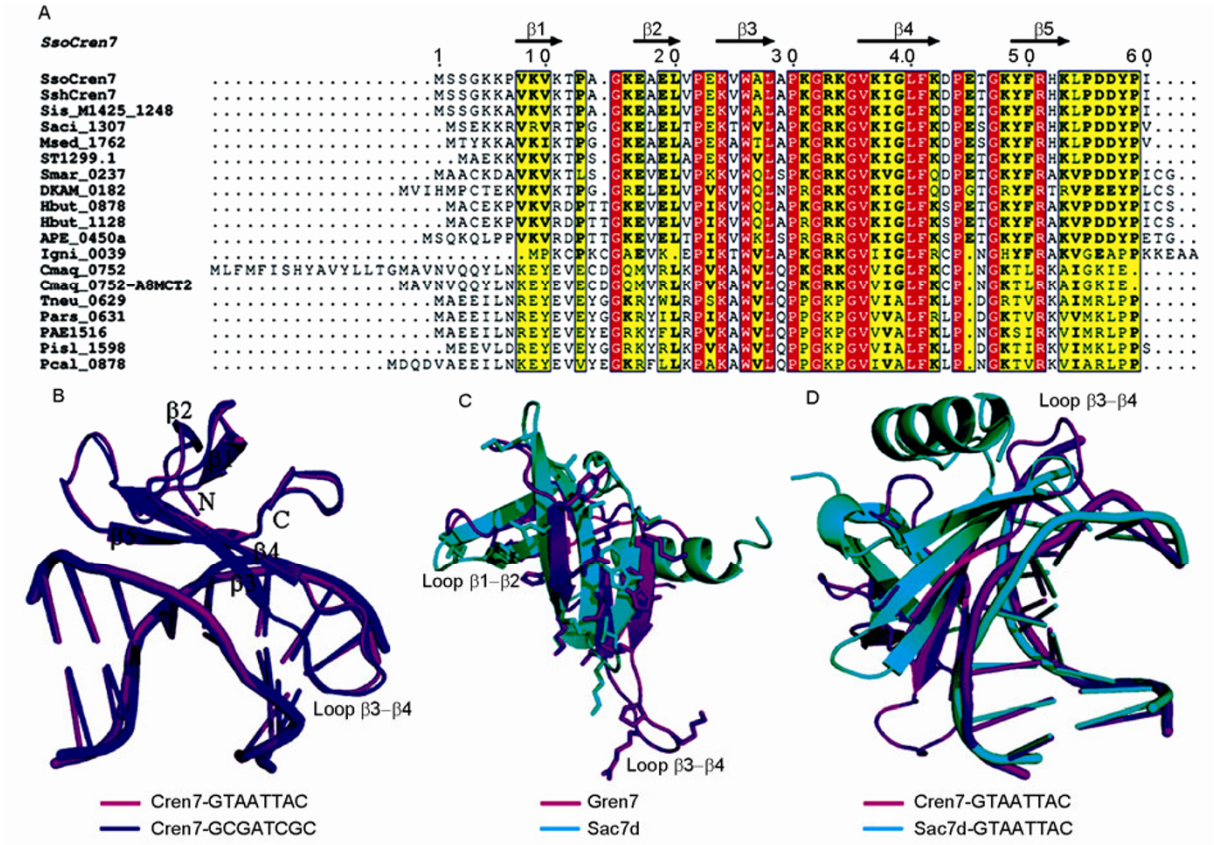

Figure 4 Structures of Cren7 in complexes with DNA. A, Sequence alignment of Cren7 homologs in crenarchaea with the secondary structural elements labeled. B, Superposition of the two Cren7-DNA complexes, fitted by common protein main-chain atoms. C, The DNA binding surfaces of Cren7 and Sac7d with the two loop regions labeled. D, Superposition of the Cren7-GTAATTAC complex (PDB code: 3LWH) and the Sac7d-GTAATTAC complex (PDB code: 1AZQ) fitted using all atoms on one of the DNA strands. The Cren7-DNA complex is shown in magenta, as in C; and the Sac7d-DNA complex is shown in cyan. The figures were generated using PyMol.

family (Figure 1) [57]. The absence of these proteins in the mesophilic euryarchaea belonging to *Halobacteria* and *Methanosarcinales* is a notable exception. Intriguingly, Sac10b homologs have also been detected in some eukarya [57]. The Sac10b proteins have a molecular mass of ~10 kD and bind DNA without apparent sequence preference. Ssh10b, a Sac10 homolog from *S. shibatae*, exists as a homodimer in solution, and is able to constrain negative DNA supercoils in a temperature-dependent fashion [58]. However, an EM study has revealed that coating of dsDNA by Sac10b does not result in significant compaction *in vitro* [49].

Native Sso10b, a Sac10b homolog from *S. solfataricus*, is acetylated at the N-terminus and on Lys16 [59]. Since acetylation was reported to decrease the affinity of the protein for DNA, the Sac10b protein family is also referred to as 'Alba', which stands for "acetylation lowers binding affinity" [59]. However, later studies showed that the difference between the acetylated and unmodified forms of the protein in DNA binding affinity was not as large as initially shown [60]. The deacetylase Sir2 associates with Sso10b *in vivo*, and the transacetylase Pat acetylates Sso10b specifically on Lys16 *in vitro* [61]. The presence of a reversible Sso10b acetylation system suggests a histone-like reversible modification of Sac10b homologs, which could be important for controlling DNA availability for replication, transcription and recombination.

Structural studies show that Sso10b, a Sac10b homolog from *S. solfataricus*, has a mixed α/β fold reminiscent of the C-terminal domain of bacterial translation initiation factor IF3 and the N-terminal domain of the nuclease DNaseI [59] (Figure 5A). Two long β-hairpin arms formed by the β3 and β4 strands extend in opposite directions from the body of the protein dimer and span a distance of ~40 Å. Two highly conserved loops are included in the highly basic surface of the central body of the protein dimer and are separated by ~20 Å (approximately the width of the DNA double helix). The loops contain two consecutive lysine residues (Lys16 and Lys17) that are both implicated in DNA binding. More recently, analysis of the crystal structure of Alba2 (Ape10b2), a Sac10b homolog from *Aeropyrum pernix*, in complex with a 16-bp duplex DNA reveals that the Alba2 dimers pack on either side of the bound dsDNA in successive minor grooves [62] (Figure 5B). The overall structure of the Alba2 subunit in the DNA complex is similar to the apo-Alba2 structure, consisting of two α-helices and four β-strands arranged in the order of β1-α1-β2-α2-β3-β4 in the primary structure. Superposition of Alba2 and DNA-bound structures showed that the extended loop connecting the β3-β4 hairpins adopts large conformational changes upon binding to DNA. However, the extended hairpin loop does not contact the bound DNA. This structural change may play an important role in oligomerization of Alba2-dimers during DNA binding to fit the incoming Alba2-dimer cooperatively without steric clashing.

Although the Sac10b proteins were long considered to be

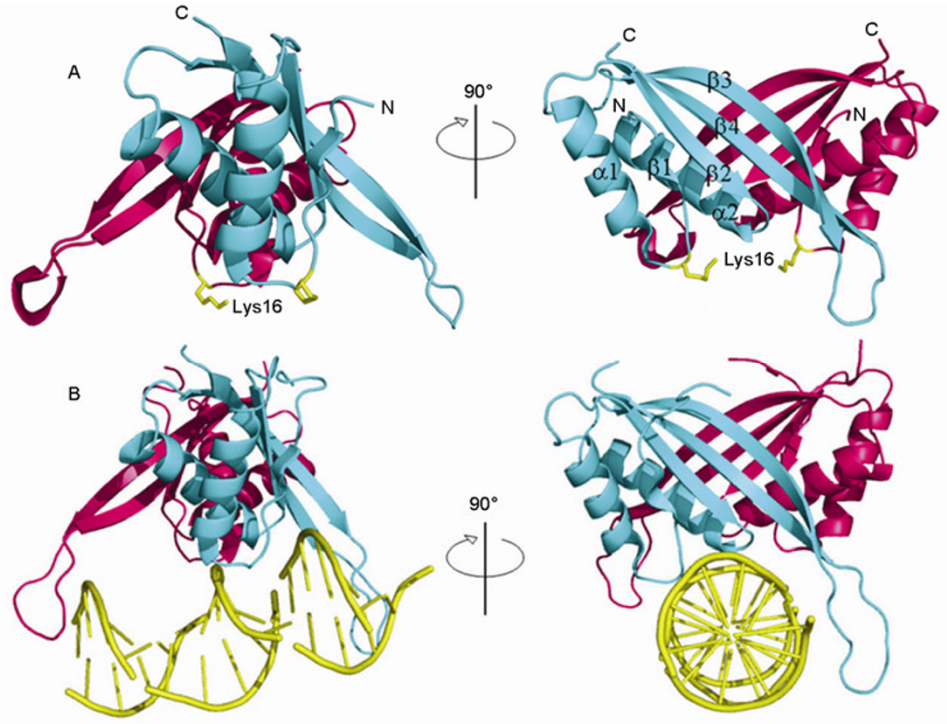

Figure 5 Structures of Sso10b and the Alba2-DNA complex. A, Structure of a homodimer of Sso10b (PDB code: 1H0X). The two monomers are shown in cyan and magenta, respectively. Residues Lys16 are marked in yellow. B, Structure of Alba2 (Ape10b) in complexes with a DNA duplex (PDB code: 3U6Y). The two monomers are shown in cyan and magenta, respectively, and DNA is shown in yellow. The figures were generated with PyMol.

chromosomal DNA-binding proteins serving a potential role in chromatin organization and DNA transactions, evidence has been accumulating that argues against DNA being the sole binding target for the proteins *in vivo* [63–65]. An early immunogold electron microscopic study shows that the *S. acidocaldarius* DBNP-B protein, which is presumably identical to Sac10b, is located exclusively in the ribosome-containing cytoplasm [63]. Ssh10b has been shown to exhibit similar affinities for dsDNA, ssDNA and ssRNA *in vitro* and co-purified with ribosomes [64]. The protein was crosslinked to RNA, instead of DNA, when the cell was irradiated with UV light. In addition, both DNase and RNase released Sso10b from cellular nucleic acid-protein complexes, suggesting that this protein is associated with both DNA and RNA *in vivo* [64]. Sequence profile searches predict that at least some members of the Sac10b family play a role in RNA metabolism [65].

The functions of the Sac10b proteins in methophilic Archaea appears to be different from those in thermophilic Archaea. Mma10b, a Sac10b homolog from *Methanococcus maripaludis*, constitutes only ~0.01% of the total cellular protein [66]. In comparison, Ssh10b accounts for 1.7% of the cellular protein [64]. The low cellular abundance suggests that Mma10b is unlikely a major architectural chromatin protein. Disruption of the gene encoding Mma10b resulted in a phenotype of poor growth in minimal medium close to the optimal growth temperature but showed no detectable effect on growth in rich medium. By contrast, attempts to delete the gene for Sis10b, a Sac10b homolog from *S. islandicus*, were unsuccessful, suggesting that the gene is essential for the growth of the organism. However, Mma10b appears to be able to bind preferentially to specific DNA sequences, raising the possibility that the protein plays a regulatory role in the expression of genes at specific loci. More recently, it was reported that Mth10b, a member of the Sac10b family from *Methanobacterium thermoautotrophicum* ΔH, binds neither DNA nor RNA *in vitro* [67]. It appears that the Sac10b proteins share an ancient origin, and have diverged greatly in function during the evolution. Whether they are recruited as chromatin proteins in some Archaea remains to be determined.

4 Conclusion and future perspectives

Despite extensive research on putative chromatin proteins from Archaea, our understanding of the chromatin architecture in this domain of life is still rudimentary. Archaea do not appear to share a unified version of chromatin. The available data suggest that two distinctly different DNA-packaging strategies, based on archaeal histones and Cren7, respectively, have evolved in Archaea. The former strategy is employed by the majority of euryarchaea, korarchaea, thaumarchaea and nanoarchaea, whereas the latter one is used by most crenarchaea. Archaeal histones are clearly capable of mediating a primary level of DNA compaction. However, it is unclear how Cren7 may play a role in chromosomal organization in crenarchaea. Obviously, more research is needed to elucidate the molecular basis of the structural role of Cren7 and other related proteins in chromosomal organization.

Over the past three decades or so, a number of small, abundant chromosomal DNA-binding proteins have been described. However, the entire landscape of chromatin proteins required for structuring and remodeling the chromatin in Archaea, especially crenarchaea, remains to be defined. As has been shown in previous studies, various phylogenetic groups in crenarchaea may employ different chromatin proteins. In addition, if archaeal histone and Cren7 are involved in the formation of basic nucleoprotein assemblies, additional chromatin proteins are presumably needed to facilitate higher-order chromosomal compaction. Therefore, searches for additional chromatin proteins are essential for chromatin modeling.

Posttranslational modification of chromatin proteins plays key roles in both chromatin packaging and regulation of DNA transactions, such as DNA replication and transcription. It has been speculated that methylation of Cren7 or Sul7d is a regulatory mechanism in DNA compaction and gene expression, presumably responding to changes in environmental stresses. Clearly, this area will attract more attention in the future.

Considerable effort has been put into the *in vitro* characterization of the proposed archaeal chromatin proteins. The combined use of traditional approaches as well as single molecule methods may aid in unraveling the features of archaeal chromatins in the cell. Clearly, exciting discoveries remain to be made that will not only increase our knowledge of chromatin structures and gene regulation in Archaea but also shed light on the evolution of chromosomal packaging in the living world.

Our work cited in this review was supported by the National Natural Science Foundation of China, Ministry of Science and Technology and Chinese Academy of Sciences. We apologize to researchers whose work was not cited because of space limitations.

1 Waters E, Hohn M, Ahel I, *et al*. The genome of *Nanoarchaeum equitans*: insights into early archaeal evolution and derived parasitism. Proc Natl Acad Sci USA, 2003, 100: 12984–12988
2 Galagan J, Nusbaum C, Roy A, *et al*. The genome of *M. acetivorans* reveals extensive metabolic and physiological diversity. Genome Res, 2002, 12: 532–542
3 Forterre P, Bergerat A, Lopez-Garcia P. The unique DNA topology and DNA topoisomerases of hyperthermophilic archaea. FEMS Microbiol Rev, 1996, 18: 237–248
4 López-García P, Forterre P. DNA topology in hyperthermophilic archaea: reference states and their variation with growth phase, growth temperature, and temperature stresses. Mol Microbiol, 1997, 23: 1267–1279
5 Forterre P, Mirambeau G, Jaxel C, *et al*. High positive supercoiling *in*

vitro catalyzed by an ATP and polyethylene glycol-stimulated topoisomerase from *Sulfolobus acidocaldarius*. EMBO J, 1985, 4: 2123–2128
6 Brochier-Armanet C, Forterre P, Gribaldo S. Phylogeny and evolution of the Archaea: one hundred genomes later. Curr Opin Microbiol, 2011, 14: 274–281
7 Sandman K, Krzychi J A, Dobrinski B, *et al*. HMf, a DNA-binding protein isolated from the hyperthermophilic archaeon *Methanothermus fervidus*, is most closely related to histones. Proc Natl Acad Sci USA, 1990, 87: 5788–5791
8 Starich M R, Sandman K, Reeve J N, *et al*. NMR structure of HMfB from the hyperthermophile, *Methanothermus fervidus*, confirms that this archaeal protein is a histone. J Mol Biol, 1996, 255: 187–203
9 Reeve J. Archaeal chromatin and transcription. Mol Microbiol, 2003, 48: 587–598
10 Cubonová L, Sandman K, Hallam S J, *et al*. Histones in Crenarchaea. J Bacteriol, 2005, 187: 5482–5485
11 Reeve J N, Bailey K A, Li W T, *et al*. Archaeal histones: structures, stability and DNA binding. Biochem Soc Trans, 2004, 32: 227–230
12 Marc F, Sandman K, Lurz R, *et al*. Archaeal histone tetramerization determines DNA affinity and the direction of DNA supercoiling. J Biol Chem, 2002, 277: 30879–30886
13 Musgrave D R, Sandman K M, Reeve J N. DNA binding by the archaeal histone HMf results in positive supercoiling. Proc Natl Acad Sci USA, 1991, 88: 10397–10401
14 Musgrave D, Forterre P, Slesarev A. Negative constrained DNA supercoiling in archaeal nucleosomes. Mol Microbiol, 2000, 35: 341–349
15 Sandman K, Grayling R A, Dobrinski B, *et al*. Growth-phase-dependent synthesis of histones in the archaeon *Methanothermus-Fervidus*. Proc Natl Acad Sci USA, 1994, 91: 12624–12628
16 Dinger M E, Baillie G J, Musgrave D R. Growth phase-dependent expression and degradation of histones in the thermophilic archaeon *Thermococcus zilligii*. Mol Microbiol, 2000, 36: 876–885
17 Sandman K, Reeve J N. Archaeal histones and the origin of the histone fold. Curr Opin Microbiol, 2006, 9: 520–525
18 Luijsterburg M S, Noom M C, Wuite G J, *et al*. The architectural role of nucleoid-associated proteins in the organization of bacterial chromatin: a molecular perspective. J Struct Biol, 2006, 156: 262–272
19 Fahrner R L, Cascio D, Lake J A, *et al*. An ancestral nuclear protein assembly: crystal structure of the *Methanopyrus kandleri* histone. Protein Sci, 2001, 10: 2002–2007
20 Heinicke I, Muller J, Pittelkow M, *et al*. Mutational analysis of genes encoding chromatin proteins in the archaeon *Methanococcus voltae* indicates their involvement in the regulation of gene expression. Mol Genet Genomics, 2004, 272: 76–87
21 Weidenbach K, Glöer J, Ehlers C, *et al*. Deletion of the archaeal histone in *Methanosarcina mazei* Gö1 results in reduced growth and genomic transcription. Mol Microbiol, 2008, 67: 662–671
22 Cheung P, Allis C D, Sassone-Corsi P. Signaling to chromatin through histone modifications. Cell, 2000, 103: 263–271
23 Kouzarides T. Histone methylation in transcriptional control. Curr Opin Genet Dev, 2002, 12: 198–209
24 Wittschieben B O, Otero G, de Bizemont T, *et al*. A novel histone acetyltransferase is an integral subunit of elongating RNA polymerase II holoenzyme. Mol Cell, 1999, 4: 123–128
25 Otero G, Fellows J, Li Y, *et al*. Elongator, a multisubunit component of a novel RNA polymerase II holoenzyme for transcriptional elongation. Mol Cell, 1999, 3: 109–118
26 DeLange R J, Green G R, Searcy D G. A histone-like protein (HTa) from *Thermoplasma acidophilum*. J Biol Chem, 1981, 256: 900-904
27 Dorman C J. Nucleoid-associated proteins and bacterial physiology. Adv Appl Microbiol, 2009, 67: 47–64
28 Le Cam E, Culard F, Larquet E, *et al*. DNA bending induced by the archaebacterial histone-like protein MC1. J Mol Biol, 1999, 285: 1011–1021
29 Brian R. Berquist, Jeetendra Soneja and Shiladitya Dassarma. Comparative genomic survey of information transfer systems in two diverse extremely halophilic Archaea, *Halobacterium* sp. strain NRC-1 and *Haloarcula Marismortui*. In: Adaptation to Life at High Salt Concentrations in Archaea, Bacteria, and Eukarya. Berlin Heidelberg: Springer Verlag, 2005. 151–183
30 Paradinas C, Gervais A, Maurizot J C, *et al*. Structure specific binding recognition of a methanogen chromosomal protein. Eur J Biochem, 1998, 257: 372–379
31 Chartier F, Laine B, Belaiche D, *et al*. Primary structure of the chromosomal protein MC1 from the archaebacterium *Methanosarcina* sp CHTI 55. Biochim Biophys Acta, 1989, 1008: 309–314
32 Grote M, Dijk J, Reinhardt R. Ribosomal and DNA binding proteins of the thermoacidophilic archaebacterium *Sulfolobus acidocaldarius*. Biochim Biophys Acta, 1986, 873: 405–413
33 Guo L, Feng Y, Zhang Z, *et al*. Biochemical and structural characterization of Cren7, a novel chromatin protein conserved among Crenarchaea. Nucleic Acids Res, 2008, 36: 1129–1137
34 Bell S, White M. Bacterial Chromatin. New York: Springer, 2010. 205–217
35 McAfee J G, Edmondson S P, Zegar I, *et al*. Equilibrium DNA binding of Sac7d protein from the hyperthermophile *Sulfolobus acidocaldarius*: Fluorescence and circular dichroism studies. Biochemistry, 1996, 35: 4034–4045
36 Guagliardi A, Napoli A, Rossi M, *et al*. Annealing of complementary DNA strands above the melting point of the duplex promoted by an archaeal protein. J Mol Biol, 1997, 267: 841–848
37 Peters W B, Edmondson S P, Shriver J W. Thermodynamics of DNA binding and distortion by the hyperthermophile chromatin protein Sac7d. J Mol Biol, 2004, 343: 339–360
38 Edmondson S P, Shriver J W. DNA-binding proteins Sac7d and Sso7d from *Sulfolobus*. Hyperthermophilic Enzymes, 2001, 334: 129–145
39 Mai V Q, Chen X, Hong R, *et al*. Small abundant DNA binding proteins from the thermoacidophilic archaeon *Sulfolobus shibatae* constrain negative DNA supercoils. J Bacteriol, 1998, 180: 2560–2563
40 Napoli A, Zivanovic Y, Bocs C, *et al*. DNA bending, compaction and negative supercoiling by the architectural protein Sso7d of *Sulfolobus solfataricus*. Nucleic Acids Res, 2002, 30: 2656–2662
41 Baumann H, Knapp S, Lundbäck T, *et al*. Solution structure and DNA-binding properties of a thermostable protein from the archaeon *Sulfolobus solfataricus*. Struct Biol, 1994, 1: 808–819
42 Gao Y G, Su S Y, Robinson H, *et al*. The crystal structure of the hyperthermophile chromosomal protein Sso7d bound to DNA. Nat Struct Biol, 1998, 5: 782–786
43 Robinson H, Gao Y G, McCrary B S, *et al*. The hyperthermophile chromosomal protein Sac7d sharply kinks DNA. Nature, 1998, 392: 202–205
44 Krueger J K, McCrary B S, Wang A H J, *et al*. The solution structure of the Sac7d/DNA complex: a small-angle X-ray scattering study. Biochemistry, 1999, 38: 10247–10255
45 Choli T, Henning P, Wittmann-Liebold B, *et al*. Isolation, characterization and microsequence analysis of a small basic methylated DNA-binding protein from the Archaebacterium, *Sulfolobus solfataricus*. Biochim Biophys Acta, 1988, 950: 193–120
46 Choli T, Wittmann-Liebold B, Reinhardt R. Microsequence analysis of DNA-binding proteins 7a, 7b, and 7e from the archaebacterium *Sulfolobus acidocaldarius*. J Biol Chem, 1988, 263: 7087–7093
47 McAfee J G, Edmondson S P, Datta P K, *et al*. Gene cloning, expression, and characterization of the Sac7 proteins from the hyperthermophile *Sulfolobus acidocaldarius*. Biochemistry, 1995, 34: 10063–10077
48 Oppermann U C, Knapp S, Bonetto V, *et al*. Isolation and structure of repressor-like proteins from the archaeon *Sulfolobus solfataricus*. Copurification of RNase A with Sso7c. FEBS Lett, 1998, 432: 141–144
49 Lurz R, Grote M, Dijk J, *et al*. Electron microscopic study of DNA complexes with proteins from the archaebacterium *Sulfolobus acidocaldarius.* EMBO J, 1986, 5: 3715–3721
50 Su S, Gao Y G, Robinson H, *et al*. Crystal structures of the chromosomal proteins Sso7d/Sac7d bound to DNA containing T-G mismatched base pairs. J Mol Biol, 2000, 303: 395–403

51 Tashiro R, Wang A H J, Sugiyama H. Photoreactivation of DNA by an archaeal nucleoprotein Sso7d. Proc Natl Acad Sci USA, 2006, 103: 16655–16659
52 Sandman K, Reeve J N. Archaeal chromation proteins: different structures but common function? Curr Opin Microbiol, 2005, 8: 656–661
53 Zhang Z, Gong Y, Guo L, *et al*. Structural insights into the interaction of the crenarchaeal chromatin protein Cren7 with DNA. Mol Microbiol, 2010, 76: 749–759
54 Luo X, Schwarz-Linek U, Botting C, *et al*. CC1, a novel crenarchaeal DNA binding protein. J Bacterial, 2007, 189: 403–409
55 Hardy C D, Martin P K. Biochemical characterization of DNA-binding proteins from *Pyrobaculum aerophilum* and *Aeropyrum pernix*. Extremophiles, 2008, 12: 235–246
56 Green G R, Searcy D G, DeLange R J. Histone-like protein in the Archaebacterium *Sulfolobus acidocaldarius*. Biochim Biophys Acta, 1983, 741: 251–257
57 Forterre P, Confalonieri F, Knapp S. Identification of the gene encoding archeal-specific DNA-binding proteins of the Sac10b family. Mol Microbiol, 1999, 32: 669–670
58 Xue H, Guo R, Wen Y, *et al*. An abundant DNA binding protein from the hyperthermophilic archaeon *Sulfolobus shibatae* affects DNA supercoiling in a temperature-dependent fashion. J Bacteriol, 2000, 182: 3929–3933
59 Wardleworth B N, Russell R J, Bell S D, *et al*. Structure of Alba: an archaeal chromatin protein modulated by acetylation. EMBO J, 2002, 21: 4654–4662
60 Jelinska C, Conroy M J, Craven C J, *et al*. Obligate heterodimerisation of the archaeal Alba2 protein with Alba1 provides a mechanism for control of DNA packaging. Structure, 2005, 13: 963–971
61 Marsh V, Peak-Chew S, Bell S. Sir2 and the acetyltransferase, Pat, regulate the archaeal chromatin protein, Alba. J Biol Chem, 2005, 280: 21122–21128
62 Tomoyuki T, Sivaraman P, Kumarevel T. Crystal structure of archaeal chromatin protein Alba2-dsDNA complex from *Aeropyrum pernix* K1. J Biol Chem, 2012, 287: 10394–10402
63 Bohrmann B, Kellenberger E, Arnold-Schulz-Gahmen B, *et al*. Localization of histone-like proteins in thermophilic Archaea by immunogold electron microscopy. J Struct Biol, 1994, 112: 70–78
64 Guo R, Xue H, Huang, L. Ssh10b, a conserved thermophilic archaeal protein, binds RNA *in vivo*. Mol Microbiol, 2003, 50: 1605–1615
65 Aravind L, Iyer L M, Anantharaman V. The two faces of Alba: the evolutionary connection between proteins participating in chromatin structure and RNA metabolism. Genome Biol, 2003, 4: R64
66 Liu Y, Guo L, Guo R, *et al*. The Sac10b homolog in *Methanococcus maripaludis* binds DNA at specific sites. J Bacteriol, 2009, 191: 2315–2329
67 Liu Y F, Zhang N, Yao H W, *et al*. Mth10b, a unique member of the Sac10b family, does not bind nucleic acid. PLoS ONE, 2011, 6: e19977